A

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	LUD5330.3DIV
First Inventor or Application Identifier	Zimmerman et al.
Title	ISOLATED PROTEINS CONTAINING PORTIONS OF FAPα AND OTHER PROTEINS
Express Mail Label No.	EI605139215US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents
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Washington, DC 20231

1. ☒ *Fee Transmittal Form *(e.g., PTO/SB/17)*
(Submit an original and a duplicate for fee processing)
2. ☒ Specification *(preferred arrangement set forth below)* — *Total Pages* 30
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Reference of Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. ☒ Drawing(s) *(35 U.S.C. 113)* — *Total Sheets* 3
4. ☒ Oath or Declaration — *Total Pages* 6
   a. ☐ Newly executed (original or copy)
   b. ☒ Copy from a prior application (37 C.F.R. § 1.63(d))
   (for continuation/divisional with Box 17 completed)
      i. ☐ DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33 (b)
5. ☒ Incorporation By Reference *(useable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be a part of the disclosure of the accompanying application and is hereby incorporated by reference therein
6. ☐ Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Copy
   b. ☒ Paper Copy (identical to computer copy)
   c. ☐ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. ☒ Assignment Papers (cover sheet & document(s))
9. ☐ 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* ☒ Power of Attorney
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. ☐ *Small Entity Statement(s) (PTO/SB/09-12) ☐ Statement filed in prior application, Status is proper and desired
15. ☐ Certified Copy of Priority Document(s)
16. ☐ Other:

NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STSTEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28)

17. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP) of prior application No:

Prior application information: 08/940,391 Examiner: A. Navarro Group / Art Unit: 1645

18. CORRESPONDENCE ADDRESS

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LUD-5330.3 DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	: Zimmerman et al
Serial No.	: Divisional of 08/940,391
Filed	: Herewith
For	: PROTEIN CONTAINING PORTIONS FAPOX AND OTHER PROTEINS
Group Art Unit	: 1645
Examiner	: M. Navarro

March 10, 1999

Hon. Commissioner of Patents
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PRELIMINARY AMENDMENT

Prior to examination please amend this application as follows:

IN THE FIGURES

Replace original figure 1 by the attached.

IN THE TITLE

Change the title to: --ISOLATED PROTEINS CONTAINING PORTIONS OF FAPα AND OTHER PROTEINS--.

IN THE SPECIFICATION

Page 6, line 3: after amino acid "optimized" add -- SEQ ID NO: 2 gives the sequence of FAP. SEQ ID NO: 3 gives the amino acid sequence of CD26. --.

Page 10, line 7: change "Dod" to -- Dod --.

Page 11, line 23: change "2812" to -- 2815 --;

line 24: change "2277" to -- 2280 --.

Page 12, line 10: change "61" to -- 51 --;

line 13: change "48" to -- 52 --;

line 21: change "eight" to -- nine --.

Page 13, Table 2:

after "WGWSYGG" (each occurrence) add -- SEQ ID NO: 4 --;

after "GTADDNV" (each occurrence) add -- SEQ ID NO: 6 --;

after "DQNHGLS" add -- SEQ ID NO: 7 --;

after "DEDHGIA" (each occurrence) add -- SEQ ID NO: 8 --;

after "FGWSYGG" add -- SEQ ID NO: 4 --;

after "DSDHSIR" add -- SEQ ID NO: 8 --;

after "FGKDYGG" (each occurrence) add -- SEQ ID NO: 5 --;

after "PTADEKI" and each occurrence of "ATADEKI" add -- SEQ ID NO: 9 --;

after "DESHYFT", "DESHYFH" and "DESHYFS" add -- SEQ ID NO: 10 --.

Page 14, line 2: change "describes" to -- described --;

line 12: change "kd" to -- kD --.

Page 19, line 19: change "due" to -- dye --.

Page 21, line 5: delete ",".

Page 26, line 17: following "library" change "," to -- . --, and add the following:

-- One can identify such enzyme inhibitors by combining a molecule which has FAP enzyme activity, such as the dimeric molecules of the invention, including dimers of SEQ ID NO: 2, with a substrate for the molecule with the enzymatic activity, as well as a substance believed to be an inhibitor. Then, one determines the activity of the molecule with enzymatic activity on its substrate, in the presence of the substance believed to be enzyme

inhibitor. If there is a decrease in activity when the test substance is present as compared to when it is absent, then the substance is an inhibitor. --.

IN THE SEQUENCES

Please see attached.

IN THE CLAIMS

Cancel claims 1-4 and 6-15 without prejudice.

Add claims 16-19 which follow:

Claim 16: The isolated protein of claim 5, wherein said non FAP protein is a CD8 protein.

Claim 17: The isolated protein of claim 5, wherein said at least one portion of a non FAP protein is an extracellular domain of a CD8 protein.

Claim 18: The isolated protein of claim 5, wherein said protein is a chimeric protein.

Claim 19: The isolated protein of claim 5, wherein said protein is a fuse in protein.

REMARKS

Entry of the foregoing is requested.

Respectfully submitted,

FULBRIGHT & JAWORSKI, L.L.P.

By__________________________
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ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF

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FELFE & LYNCH

Pauline Smith
(Printed name)

(Signature)

RELATED APPLICATION

This application is a continuation-in-part of Serial No. 08/230,491, filed April 20, 1994, now pending and incorporated by reference.

FIELD OF THE INVENTION

This invention relates to certain molecules associated with cancer tissues and reactive tumor stromal cells. More particularly, it relates to fibroblast activation protein alpha ("FAPα" hereafter) molecules. A monomeric form of the molecule has previously been identified immunochemically, but nucleic acid molecules coding for it had not been isolated or cloned nor have dimers been identified. These, inter alia, are features of the invention. The monomeric protein has a molecular weight of from about 88 to about 95 kilodaltons as determined by SDS-PAGE of boiled samples. The dimer has a molecular weight of about 170 kilodaltons as determined by SDS-PAGE of unboiled samples. FAPα is characterized by a number of features and properties which are shared by and characteristic of membrane bound enzymes, suggesting very strongly that it, too, is a membrane bound enzyme. The nucleic acid molecules, which are a key part of the invention, are useful both as probes for cells expressing FAPα, and as starting materials for recombinant production of the protein. The FAPα protein can then be used to produce monoclonal antibodies specific for the protein and are thus useful diagnostic agents themselves.

They also have additional uses, including uses related to enzymatic functions, as described herein.

BACKGROUND AND PRIOR ART

The invasive growth of epithelial cancers is associated with characteristic cellular and molecular changes in the supporting stroma. For example, epithelial cancers induce the formation of tumor blood vessels, the recruitment of reactive tumor stromal fibroblasts, lymphoid and phagocytic infiltrates, the release of peptide mediators and proteolytic enzymes, and the production of an altered extracellular matrix (ECM). See, e.g., Folkman, Adv. Cancer Res. 43: 175-203 (1985); Basset et al., Nature 348: 699-704 (1990); Denekamp et al., Cancer Metastasis Rev. 9: 267-282 (1990); Cullen et al., Cancer Res. 51: 4978-4985 (1991); Dvorak et al., Cancer Cells 3: 77-85 (1991); Liotta et al., Cancer Res. 51: 5054s-5059s (1991); Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989). A highly consistent molecular trait of the stroma in several common histologic types of epithelial cancers is induction of the fibroblast activation protein (FAPα), a cell surface glycoprotein with an observed M_r of 95,000 originally discovered with a monoclonal antibody, mAb F19, raised against proliferating cultured fibroblasts. See Rettig et al., Cancer Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. USA 87: 7235-7239 (1990); Rettig et al., Cancer Res. 53: 3327-3335 (1993). Each of

these four papers is incorporated by reference in its entirety.

Immunohistochemical studies such as those cited supra have shown that FAPα is transiently expressed in certain normal fetal mesenchymal tissues but that normal adult tissues are generally FAPα⁻. Similarly, malignant epithelial, neural and hematopoietic cells are generally FAPα⁻. However, most of the common types of epithelial cancers, including >90% of breast, lung, skin, pancreas, and colorectal carcinomas, contain abundant FAPα⁺ reactive stromal fibroblasts. Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990). The FAPα⁺ tumor stromal fibroblasts almost invariably accompany tumor blood vessels, forming a distinct cellular compartment interposed between the tumor capillary endothelium and the basal aspect of malignant epithelial cell clusters. While FAPα⁺ stromal fibroblasts are found in both primary and metastatic carcinomas, benign and premalignant epithelial lesions, such as fibroadenomas of the breast and colorectal adenomas only rarely contain FAPα⁺ stromal cells. In contrast to the stroma-specific localization of FAPα in epithelial neoplasms, FAPα is expressed in the malignant cells of a large proportion of bone and soft tissue sarcomas. (Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988)). Finally, FAPα⁺ fibroblasts have been detected in the granulation tissue of healing wounds (Garin-Chesa et al., <u>supra</u>). Based on the restricted distribution pattern of FAPα in normal tissues and its uniform expression in the supporting stroma of many epithelial cancers,

clinical trials with ^{131}I-labeled mAb F19 have been initiated in patients with metastatic colon cancer (Welt et al., Proc. Am. Assoc. Cancer Res. 33: 319 (1992); Welt et al. J. Clin. Oncol. 12: 1561-1571 (1994)) to explore the concept of "tumor stromal targeting" for immunodetection and immunotherapy of epithelial cancers.

Rettig et al., Int. J. Cancer 58: 385-392 (1994), incorporated by reference, discusses the FAPα molecule and its features. Rettig et al postulate that FAPα is found in high molecular weight complexes in excess of 400 kilodaltons, but do not discuss the possibility of dimeric molecules, nor does the paper elaborate on the specific enzymatic properties of the molecule.

The induction of FAPα^+ fibroblasts at times and sites of tissue remodeling during fetal development, tissue repair, and carcinogenesis is consistent with a fundamental role for this molecule in normal fibroblast physiology. Thus, it is of interest and value to isolate and to clone nucleic acid molecules which code for this molecule. This is one aspect of the invention, which is described in detail together with other features of the invention, in the disclosure which follows. Further aspects of the invention include the dimeric FAPα molecules, and the exploitation of the properties of these molecules. These features are also elaborated upon hereafter.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 compares the deduced amino acid sequence for FAPα, and the known sequence of CD26. The alignment has been optimized.

Figures 2A-2H, inclusive, display immunohistochemical detection of FAPα and CD26 in various tissues. In figures 2A and 2B, breast cancer is studied, for FAPα (figure 2A), and CD26 (figure 2B). In figures 2C and 2D, malignant fibrous histiocytoma is studied, for FAPα (figure 2C), and CD26 (figure 2D). Dermal scar tissue is examined in figures 2E (FAPα), and 2F (CD26). Renal cell carcinoma is studied in figure 2G (FAPα), and 2H (CD26).

Figure 3 presents some of the data generated in experiments which showed that FAPα had extracellular matrix (ECM) protein degrading activity. When zymographic detection of gelatin degrading extracts of 293-FAP was carried out, the active substance was found to have a molecular weight of about 170 kD, via SDS-PAGE, using unboiled samples to preserve enzyme activity.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Fibroblast cell line WI-38 had been observed, previously, to react with mAb F19 (Rettig et al., Canc. Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990);

Rettig et al., Canc. Res. 53: 3327-3335 (1993)). It was used in the experiments which follow.

A cDNA library was prepared from WI-38, using well known techniques and commercially available materials. Specifically, the library was constructed in expression vector pCDNAI, using the Fast Track mRNA isolation kit, and Librarian cDNA phagemid system. Once the library was prepared, the vectors were electroporated into cell line E. coli MC 1061/P3. The pCDNAI expression vector contains an antibiotic resistance gene, so the E. coli were selected via antibiotic resistance. The colonies which were resistant were then used in further experiments. The plasmid DNA from the colonies was obtained via alkaline lysis and purification on $CsCl_2$, in accordance with Sambrook et al, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 2d Ed. 1989). The technique is well known to the art, but is incorporated by reference herein.

Once the plasmid DNA was isolated, it was used to transfect COS-1 cells, which were then cultured for forty-eight hours, after which these were tested with antibody coated dishes. The mAbs used included F19, as described by Rettig et al., (1986), supra, which is incorporated by reference in its entirety. As COS-1 cells are normally $FAP\alpha^-$, any positive results indicated the presence of the coding sequence. The immunoselection protocol was that of Aruffo et al., Proc. Natl. Acad. Sci USA 84: 3365-3369 (1987), incorporated by reference herein.

Plasmid DNA from positive clones was recovered, in accordance with Hirt, J. Mol. Biol. 26: 365-369 (1967), reintroduced into E. coli MC 1061/P3, and reselected in COS-1 cells.

The protocol presented herein was followed for four rounds. After this, the plasmid DNA of 50 isolated bacterial colonies was purified, using the Qiagen plasmid kit. Of the colonies, 27 clones were found to contain identical 2.8 kb inserts, as determined by EcoRI restriction enzyme mapping. Several of these were found to contain FAPα-specific cDNA as determined by transient expression in COS-1 cells and direct immunofluorescence staining with mAb F19. One of these clones, i.e., "pFAP.38" was selected for further study, as elaborated upon infra.

Example 2

Once pFAP.38 had been identified, it was tested together with a vector coding for known cell surface marker CD26 ("pCD26"), as well as with control vector pCDNA I.

In these experiments, COS-1 cells were transfected with one of pFAP.38, pCD26, or pCDNAI. After forty-eight hours, the transfectants were tested, using the well known MHA rosetting assay for cell surface antigen expression. In these experiments, mAb F19, which is FAPα specific, was used, together with mAb EF-1, which is CD26 specific. Also used were four other FAPα specific mAbs, i.e., FB23, FB52, FB58 and C48. Also tested were two cancer cell lines, which are known to react with mAb F19 (SW872 liposarcoma), or EF-1 (SK-OV6 ovarian cancer). The results are set

forth in Table 1, which follows.

Table 1. Cell surface expression of multiple FAPα epitopes and CD26 in human cells and COS-1 cell transfectants

	Cell surface antigen expression					
Target cell	F19	FB23	FB52	FB58	C48	EF-1
<u>Human cells</u>						
SW872 liposarcoma	>95%	>95%	>95%	>95%	>95%	-
SK-OV6 ovarian cancer	-	-	-	-	-	>95%
<u>COS-1 transfectants</u>						
COS·pCDNAI control	-	-	-	-	-	-
COS·pFAP 38	40%	30%	40%	20%	20%	-
COS·pCD26	-	-	-	-	-	40%

<u>Example 3</u>

Immunoprecipitation studies were then carried out to identify the antigen being targeted by the antibodies.

Cells were metabolically labelled with Trans ^{35}S-label, (ICN), extracted with lysis buffer (0.01 M Tris-HCl/0.15 M NaCl/0.01 M $MgCl_2$/0.5% Nonidet P-40/aprotinin (20 ug/ml)/2 mM phenylmethylsulfonyl fluoride), and then immunoprecipitated. The protocols used are all well known, as will be seen by reference to Rettig et

al., Canc. Res. 53: 3327-3335 (1993); and Fellinger et al., Canc. Res. 51: 336-340 (1991), the disclosures of which are all incorporated by reference in their entirety. Precipitating mAbs were negative control mouse Ig, mAb F19, or EF-1. Control tests were carried out with mock transfected COS-1 cells. Following immunoprecipitation, the immunoprecipitates were boiled in extraction buffer and separated by $NaDOdSO_4$/PAGE, under reducing conditions. In some experiments, an additional test was carried out to determine whether or not the immunoprecipitated material was glycosylated. In these experiments, cell extracts were fractionated with Con A-SEPHAROSE prior to immunoprecipitation. Following immunoprecipitation, but prior to fractionation on $NaDodSO_4$/PAGE, these precipitates were digested with N-Glycanase.

The results showed that, in COS-1 cells, pFAP.38 directs expression of an 88 kd protein species (as determined via SDS-PAGE), which is slightly smaller than the 95 kd FAPα species produced by SW872, or cultured fibroblasts. Digestion with N-Glycanase produced peptides of comparable size (i.e., 74 kd versus 75 kd), showing that the glycosylation of the FAPα protein in COS-1 cells is different than in the human cell lines.

Example 4

Classic Northern blot analysis was then carried out, using the mRNA from $FAP\alpha^+$ fibroblast cell lines WI-38 and GM 05389, and $FAP\alpha^-$ ovarian cancer cell line SK-OV6. Using the procedures of Sambrook

et al., supra, five micrograms of mRNA from each cell line were tested. The probes used were ^{32}P labelled, and were prepared from a 2.3 kb ECO I fragment of pFAP.38, a 2.4 kb Hind III fragment of CD26, and a 1.8 kb BamHI fragment of γ-actin cDNA. These fragments had been purified from 1% agarose gels.

The extracts of FAPα^+ fibroblast strains showed a 2.8 kb FAP mRNA species, but extracts of SK-OV6 do not. A γ-actin mRNA species (1.8 kb), was observed in all species.

Example 5

The cDNA identified as coding for FAPα was subjected to more detailed analysis, starting with sequencing. The classic Sanger methodology, as set forth in Proc. Natl. Acad. Sci. USA 74: 5463-5467 (1977), was used to sequence both strands of the cDNA. Once this was secured, an amino acid sequence was deduced therefrom. This information is presented in SEQ ID NO: 1. The sequence was then compared to the known amino acid sequence of CD26 (Morimoto et al., J. Immunol. 143: 3430-3437 (1989)). Figure 1 presents the comparison, using optimized sequence alignment. Any gaps in the comparison are indicated by asterisks, while identical amino acids are shown by dashes in the CD26 sequence. A hydrophobic, putative transmembrane sequence is double underlined, while potential N-glycosylation sites are single underlined.

The sequence analysis shows a 2812 base pair insert, wherein 2277 base pairs constitute the open reading frame. This ORF

extends from start codon ATG at nucleotide 209, to stop codon TAA at 2486.

The deduced polypeptide is 760 amino acids long, and has a molecular weight of 87,832. In contrast, N-Glycanase digested, immunopurified FAPα was reported to have an estimated M_r of 75,000 on $NaDodSO_4$/PAGE (Rettig et al., Canc. Res. 53: 3327-3335 (1993)).

A GenBank data base search was carried out. The most closely related genes found were those encoding dipeptidyl peptidase IV homologues (DPPIV; EC 3.4.14.5), with human DPPIV (also known as T-cell activation antigen CD26), showing 61% nucleotide sequence identity, and 48% amino acid sequence identity.

The second set of related genes are human, rat, and bovine homologues of DPPX, a gene of unknown function widely expressed in brain and other normal tissues. The predicted human DPPX gene product shows about 30% amino acid sequence identity with FAPα and CD26. The FAPα molecule exhibits structural features typical of type II integral membrane proteins, including a large COOH-terminal extracellular domain, a hydrophobic transmembrane segment, and a short cytoplasmic tail. The putative extracellular domain contains five potential N-glycosylation sites, eleven cysteine residues (eight of which are conserved between FAPα and CD26), and three segments corresponding to highly conserved catalytic domains characteristic of serine proteases, such as DPPIV. These conserved sequences are presented in Table 2, which follows. Comparisons to DPPIV and DPPX were made via Morimoto et al., <u>supra</u>; Wada et al.,

Proc. Natl. Acad. Sci. USA 89: 197-201 (1992); Yokotani et al., Human Mol. Genet. 2: 1037-1039 (1993).

Table 2. Putative catalytic domains of FAPα, DPPIV and DPPX.

	624	702	734
Human FAPα	....WGWSYGG.......	.GTADDNV........	DQNHGLS....
Human DPPIV	....WGWSYGG.......	.GTADDNV........	DEDHGIA....
Mouse DPPIV	....WGWSYGG.......	.GTADDNV.6.....	DEDHGIA....
Rat DPPIV	.4..WGWSYGG.......	.GTADDNV........	DEDHGIA.... 8
Yeast DPPIV	....FGWSYGG.......	.GTGDDNV........	DSDHSIR....
Human DPPX	....FGKDYGG.......	.PTADEKI........	DESHYFT....
Rat DPPX	....FGKDYGG 5......	.ATADEKI 9......	DESHYFH... 10
Bovine DPPX	....FGKDYGG.......	.ATADEKI........	DESHYFS....

Example 6

An additional set of experiments were carried out to determine whether FAPα related sequences are present in non-human species. To do so, human, mouse, and Chinese hamster genomic DNA was

digested using restriction enzymes, and tested, via Southern blotting, using the 2.3 kb fragment, labelled with ^{32}P, describes supra. Hybridization was carried out using stringent washing conditions (0.1 x SSC, 0.1% $NaDodSO_4$, 68°C). Cross-hybridization was readily observed with both the mouse and hamster DNA, suggesting the existence of highly conserved FAPα homologues. In control experiments using the CD26 cDNA fragment described supra, no evidence of cross hybridization was observed.

Example 7

The CD26 molecule shares a number of biochemical and serological properties with FAPß, which is a previously described, FAPα associated molecule having a molecular weight of 105 kd, and is found on cultured fibroblasts and melanocytes (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). Cotransfection experiments were carried out to determine whether FAPß is a CD26 gene product. To test this, the same protocols were used which were used for transfection with pFAP.38 or pCD26, as described supra, but using the two vectors. The results presented supra showed that cotransfection efficiency was about 40% for each vector, so about 10-20% of cell should be cotransfected.

Following cotransfection, the COS-1 cells were Trans ^{35}S-labeled, as described supra, then lysed, also as described supra.

The resulting cell extracts were separated on Con A SEPHAROSE, and the antigen (FAPα and/or CD26) were recovered in the Con A-

bound fraction. The bound fraction was eluted with 0.25 M α-D-mannopyranoside. Immunoprecipitation was then carried out, as described supra, and the precipitates were separated on $NaDodSO_4$/PAGE, also as discussed supra.

Those cells transfected only with pFAP.38 produced FAPα, but not FAPß (determined from mAb F19 immunoprecipitates). They also produce no CD26 antigen (tested with EF-1). Those cells transfected with pCD26 alone produce CD26 but no FAPα. Cotransfectants produce CD26 and FAPα/FAPß heteromers, as determined in the mAb F19 precipitates. This result provides direct evidence that FAPß is a CD26 gene product.

Example 8

It has been observed previously that some cultured human cell types coexpress FAPα and CD26, and show FAPα/CD26 heteromer formation. *In vivo* distribution patterns of FAPα and CD26, however, as determined in previous immunohistochemical studies, appeared to be non-overlapping. (See Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7329 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Stein et al., in Knapp et al., eds. Leukocyte typing IV-white cell differentiation antigens, pp 412-415 (Oxford University Press, N.Y. 1989), pp. 412-415; Möbious et al., J. Exp. Immunol. 74: 431-437 (1988)). In view of the potential significance of FAPα/CD26 coassociation, tissue distribution was

reexamined, via side by side immunohistochemical staining of normal tissues and lesional tissues known to contain FAPα$^+$ fibroblasts or FAPα$^+$ malignant cells.

To test the samples, they were embedded in OCT compound, frozen in isopentane precooled in liquid nitrogen, and stored at -70°C until used. Five micrometer thick sections were cut, mounted on poly-L-lysine coated slides, air dried, and fixed in cold acetone (4°C, for 10 minutes). The sections were then tested with mAbs (10-20 ug/ml), using the well known avidin-biotin immmuno-peroxidase method, as described by, e.g., Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Garin-Chesa et al., Am. J. Pathol. 142: 557-567.

The results are shown in figure 2. Breast, colorectal, pancreas and lung carcinomas showed strong expression of FAPα and no CD26 was found (see figures 2A and 2B). Five FAPα$^+$ sarcomas, including malignant fibrous histiocytoma (figures 2C and 2D), were tested, and there was no expression of CD26. Examination of reactive fibroblasts of healing dermal wounds (figures 2E, 2F), showed abundant expression of both FAPα and CD26. The three renal carcinomas tested (figures 2G, 2H), showed expression of CD26 in malignant epithelium. FAPα was absent from malignant epithelial cells, and showed low expression in the stroma of these carcinomas.

Example 9

A mammalian cell line, transfected with a FAPα encoding cDNA, was prepared.

Human embryonic kidney cell line 293 is well known and widely available from, e.g., the American Type Culture Collection.

Samples of 293 were maintained, in an incubator, at 37°C, in an atmosphere of 95% air, and 5% CO_2. The cells were cultured in a 50:50 mixture of Dulbecco's modified minimal essential medium and Ham's F12 medium, augmented with 10% fetal bovine serum, penicillin and streptomycin. Following the procedures described by Ustar et al., Eur. Mol. Biol. J. 1991, and/or Park et al., J. Biol. Chem. 169: 25646-25654 (1994), both of which are incorporated by reference, cDNA for FAPα (i.e., SEQ ID NO: 1), was transfected into the 293 cells. Details of the cDNA vector are provided, supra (pFAP.38). Transfectants were selected for resistance to antibiotics (200 ug/ml Geneticin), and were then maintained in selection medium, containing Geneticin.

Individual colonies of resistant cells were picked, grown to confluence in 6 well tissue culture plates, and were tested for FAPα expression in an immunofluorescence assay (IFA), using FAPα specific monoclonal antibody F19 as described supra.

Those colonies which expressed FAPα were expanded, and monitored by indirect IFA and cytofluorometric analysis, also as set forth, supra.

The IFAs were positive for the transfectants, referred to hereafter as cell line 293-FAP, but were negative for parental line 293.

Example 10

In order to confirm that recombinant FAPα was, in fact, being produced, a series of immunoprecipitation experiments were carried out. These followed the methods of Park, et al., supra, and Rettig et al., Canc. Res. 53: 3327-3335 (1993), both of which are incorporated by reference. Essentially, ^{35}[S] methionine labelled cell extracts were combined with monoclonal antibody F19, in the manner described supra. Precipitates were then boiled in extraction buffer and run on SDS-PAGE gels, using, as a negative control, mouse IgG1. Both cell line 293-FAP, and non transfected line 293 were tested. The results indicated clearly, that recombinant FAPα was produced by the transfected cell line 293-FAP. This was determined by immunoprecipitation analyses, using FAPα specific monoclonal antibody F19.

Example 11

The ability to produce recombinant FAPα permitted further study of the molecule's properties. Specifically, given the structural features outlined in the prior examples, experiments were designed to determine if FAPα possesses enzymatic activities. The experiments were designed to test whether or not FAPα had

extracellular matrix (ECM) protein degrading activity.

Extracts of 293-FAP cells were prepared, using an extraction buffer (0.15M NaCl, 0.05M Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 percent Triton X-114), were cleared by centrifugation (4,000xg, 10 minutes at 4°C), and phase partitioned at 37°C for 10-20 minutes. This was followed by further centrifugation (4000xg, 20 minutes at 20-25°C). Detergent phases were diluted with buffer (0.15 M NaCl, 0.05 M Tris-HCl pH 7.4, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.75% Empigen BB), and separated on concanavalin A-Sepharose following Rettig et al., <u>supra</u>. Any concanavalin A bound fractions were eluted with 0.25M methyl-α-D-mannopyranoside in elution buffer 0.15 M NaCl,0.05 M Tris-HCl, pH 7.4, 5mM $CaCl_2$, 5 mM $MgCl_2$, 0.1% Triton X-100), mixed with zymography sample buffer (0.25 M Tris-HCl, pH 6.8, 8% SDS, 40% glycerol, 0.01% bromophenol blue), at a 3:1 ratio, and used for further analysis.

Aliquots of sample were loaded onto polyacrylamide gels containing 0.1% of either of gelatin or casein. Electrophoresis was then carried out in a Biorad Mini-Protein II system, at 20 mA constant current for 1.5 - 2 hours, until the bromophenol blue due fronts of samples had reached the lower end of the gel. The gel was removed and incubated for one hour at 20-25°C in a 2.5% aqueous solution of Triton X-100 on a rotary shaker. The Triton X-100 solution was decanted, and replaced with enzyme buffer (0.05M Tris-HCl, pH 7.5, 0.2M NaCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$, 0.02% Brij 35). The gel was then incubated at 37°C or 41°C, followed by staining or

destaining at room temperature. Gels were stained with 0.5% of Coomassie Brilliant Blue G-250 in an aqueous solution of 30% methanol and 10% acetic acid for 15, 30, and 60 minutes, respectively. Subsequently, gels were incubated for 15 minutes in an aqueous solution of 30% CH_3OH and 5% glycerol, followed by drying between sheets of cellophane.

Gelatinase activity was evaluated in accordance with Kleiner et al., Anal. Biochem. 218: 325-329 (1994), incorporated by reference in its entirety. This is a routine assay used to determine whether or not a protease capable of digesting gelatin is present. Labelled molecular weight standard were run on the same gels, under reducing conditions, for molecular weight determinations.

Proteolytic activity for defined amino acid sequence motifs were tested, using a well known membrane overlay assay. See Smith et al, Histochem. J. 24(9): 637-647 (1992), incorporated by reference. Substrates were Ala-Pro-7-amino-4-trifluoromethyl coumarin, Gly-Pro-7-amino-4-trifluoromethyl coumarin, and Lys-Pro-7-amino-4-triflurometyl coumarin.

The results of these experiments are depicted, in part, in figure 3. This figure shows zymographic detection of gelatin degrading activity, in the cell extracts. See Kleiner et al., supra. A protein species of approximately 170 kilodaltons, as determined by SDS-PAGE, was observed to have gelatin degrading activity. This species, which was found in the 293-FAP cell line,

but not in untransfected 293 cells, is thus identified as FAPα. The molecular weight is consistent with a dimer, i.e., a dimeric FAPα molecule.

The proteolytic activity described herein where gelatin is the substrate, was not observed when casein was the substrate.

Example 12

Further studies were then undertaken in order to characterize the 170 kD FAPα dimer further. Specifically, the experiments described in example 11 were repeated, except that 5% of 2-mercaptoethanol or 5 um iodoacetamide was added to the extracts prior to SDS-PAGE, or ethylenediamine N,N,N',N'-tetraacetic acid (10 mM) was added to the incubation buffer used for gelatin zymography. None of these treatments abolished the enzymatic activity. In contrast, heating at 100°C for five minutes prior to SDS-polyacrylamide gel electrophoresis abolished the gelatin-degrading activity.

Further work, using a membrane overlay assay, described by, e.g., Smith et al., Histochem J. 24(9): 643-647 (1992), incorporated by reference, revealed that the FAPα dimers were able to cleave all of the Ala-Pro, Gly-Pro, and Lys-Pro dipeptides tested.

In further experiments, a fusion protein was produced which comprised the extracellular domains of both FAPα and murine CD8 proteins. This chimeric protein was produced in a baculovirus system in insect cells. The chimeric protein exhibited the same

enzymatic activity as FAPα, using the model discussed <u>supra</u>.

The foregoing examples describe an isolated nucleic acid molecule which codes for fibroblast activating protein alpha ("FAPα"), as well as dimeric forms of the molecule, and uses thereof. The expression product of the sequence in COS-1 is a protein which, on SDS-PAGE of boiled samples, shows a molecular weight of about 88 kd. Deduced amino acid sequence, as provided in SEQ ID NO: 1, for one form of the molecule, yields a molecular weight of about 88 kd.

It should be noted that there is an apparent discrepancy in molecular weight in that the COS-1 isolate is glycosylated, while molecular weight from deduced amino acid sequences does not account for glycosylation. Membrane proteins are known to exhibit aberrant migration in gel systems, however, which may explain the difference observed here.

Also a part of the invention are chimeric and fusion proteins, which comprise a portion of FAPα which contain the molecule's catalytic domain, and additional, non FAPα components. The FAPα catalytic domain <u>per se</u> is also a part of the invention.

It is to be understood that, as described, FAPα may be glycosylated, with the type and amount of glycosylation varying, depending upon the type of cell expressing the molecule. The experiment described herein shows this. This is also true for the dimeric form of the molecule, first described herein, having a molecular weight of about 170 kilodaltons as determined by SDS-PAGE of unboiled samples.

The invention also comprehends the production of expression vectors useful in producing the FAPα molecule. In their broadest aspect, these vectors comprise the entire FAPα coding sequence or portions thereof, operably linked to a promoter. Additional elements may be a part of the expression vector, such as protein domains fused to the FAPα protein or protein portions ("fusion protein") genes which confer antibiotic resistance, amplifiable genes, and so forth.

The coding sequences and vectors may also be used to prepare cell lines, wherein the coding sequence or expression vector is used to transfect or to transform a recipient host. The type of cell used may be prokaryotic, such as E. coli, or eukaryotes, such as yeast, CHO, COS, or other cell types.

The identification of nucleic acid molecules such as that set forth in SEQ ID NO: 1 also enables the artisan to identify and to isolate those nucleic acid molecules which hybridize to it under stringent conditions. "Stringent condition" as used herein, refers to those parameters set forth supra, whereby both murine and hamster sequences were also identified. It will be recognized by the skilled artisan that these conditions afford a degree of stringency which can be achieved using parameters which vary from those recited. Such variance is apprehended by the expression "stringent conditions".

The ability of nucleic acid molecules to hybridize to complementary molecules also enables the artisan to identify cells which express FAPα, via the use of a nucleic acid hybridization

assay. One may use the sequences described in the invention to hybridize to complementary sequences, and thus identify them. In this way, one can target mRNA, e.g., which is present in any cell expressing the FAPα molecule.

It is of course understood that the nucleic acid molecules of the invention are also useful in the production of recombinant FAPα, in both monomeric and dimeric form. The examples clearly show that host cells are capable of assembling the dimeric forms. The recombinant protein may be used, e.g., as a source of an immunogen for generation of antibodies akin to known mAb F19, and with the same uses. Similarly, the recombinant protein, and/or cells which express the molecule on their surface, may be used in assays to determine antagonists, agonists, or other molecules which interact with the FAPα molecule. Such molecules may be, but are not necessarily limited to, substrates, inhibiting molecules, antibodies, and so forth. This last feature of the invention should be considered in light of the observed structural resemblances to membrane bound enzymes. This type of molecule is associated with certain properties which need not be described in detail here. It will suffice to say that inhibition or potentiation of these properties as associated with FAPα is a feature of this invention. For example, one may identify substrates or the substrate for FAPα molecules, via the use of recombinant cells or recombinant FAPα per se. The substrates can be modified to improve their effect, to lessen their effect, or simply to label them with detectable signals so that they can be

used, e.g., to identify cells which express FAPα. Study of the interaction of substrate and FAPα, as well as that between FAPα and any molecule whatsoever, can be used to develop and/or to identify agonists and antagonists of the FAPα molecule.

Also a feature of the invention are isolated, dimeric FAPα molecules which have a molecular weight of about 170 kilodaltons as determined by SDS-PAGE, their use as an enzymatic cleaving agent, and other uses as are described herein. Enzymatically active forms of FAPα may also be produced as recombinant fusion proteins, comprising the catalytic domain of FAPα and other protein domains with suitable biochemical properties, including secretory signals protease cleavage sites, tags for purification, and other elements known to the artisan. The fact that FAPα has particular properties, as described herein, permits the identification of the molecule on cells expressing them. In turn, because the FAPα molecule is associated with tumors and tumor stromal cells, targeting of FAPα with therapeutic agents serves as a way to treat cancerous or precancerous condition, by administering sufficient therapeutic agent to alleviate cancer load.

The experiments showing the proteolytic properties of FAPα lead to yet a further aspect of the invention. It is well known that proteases which degrade extracellular matrix, or "ECM" proteins have an important role on certain aspects of tumor growth, including their effect on tumor cell invasion, tumor blood vessel formation (i.e., neoangiogenesis), and tumor metastasis. Collagens are of special interest vis-a-vis the substrates of proteases, as

the collagens are an important part of the ECM. The fact that FAPα digests ECM suggests a therapeutic role for inhibitors of the molecule. "Inhibitors", as used herein, refers to molecules which interfere with FAPα enzyme function. Specifically excluded from such inhibitors is the monoclonal antibody F19. This mAb is known to bind to but not inhibit the enzyme function of FAPα, and hence it is not an inhibitor. The art is quite well versed with respect to monoclonal antibodies which both bind to and inhibit enzymes. Further examples of such inhibitors would include, e.g., substrate derivatives, such as modified collagen molecules, which interfere with the active site or sites of the FAPα molecule. Other suitable inhibitors will be apparent to the skilled artisan, and need not be listed here. In addition, the recombinant FAPα proteins and FAPα-transfected cell lines described <u>supra</u> can be employed in an enzymatic screening assay, using the substrate described <u>supra</u> or other suitable substrates, to identify inhibitors from any compound library,

Other aspects of the invention will be clear to the skilled artisan, and need not be set forth here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

<u>We claim</u>:

1. Isolated, dimeric FAPα molecule, having a molecular weight of about 170 kilodaltons as determined by SDS-PAGE, wherein said dimeric FAPα molecule is capable of degrading extracellular matrix proteins.

2. The dimeric FAPα molecule of claim 1, wherein each monomer of said dimeric FAPα molecule consists of the amino acid sequence of SEQ ID NO: 2.

3. The dimeric FAPα molecule of claim 1, produced recombinantly.

4. The dimeric FAPα molecule of claim 3, produced by a eukaryotic cell.

5. Isolated protein consisting of:
   (i) the FAPα catalytic domain, and
   (ii) at least one portion of a non FAPα protein.

6. Method for cleaving a terminal dipeptide of formula Xaa-Pro from a molecule, comprising contacting said molecule with a second molecule, said second molecule having FAPα enzymatic activity.

7. The method of claim 6, wherein said second molecule is isolated, dimeric FAPα.

8. The method of claim 6, wherein said second molecule comprises an FAPα catalytic domain.

9. Method for identifying an enzyme inhibitor, comprising combining:

(i) a molecule having FAPα enzymatic activity;

(ii) a substrate for said molecule;

(iii) a substance believed to be an enzyme inhibitor; and

(iv) determining activity of (i) on (ii), wherein a decrease in activity when (iii) is present as compared to activity when (iii) is absent indicates that said substance is an enzyme inhibitor.

10. The method of claim 9, wherein said molecule is isolated dimeric FAPα.

11. The method of claim 9, wherein said molecule comprises an FAPα catalytic domain.

12. Method for treating a subject with a pathological condition characterized by FAPα expression, comprising administering to said subject an amount of a FAPα inhibitor sufficient to inhibit enzyme activity of FAPα.

13. The method of claim 12, wherein said inhibitor is a monoclonal antibody.

14. The method of claim 12, wherein said inhibitor is a collagen derivative.

15. The method of claim 12, wherein said pathological condition is a cancer.

ABSTRACT OF THE DISCLOSURE

The invention involves dimeric forms of the protein known as fibroblast activation protein alpha, or "FAPα" and its uses.

FIG. 1

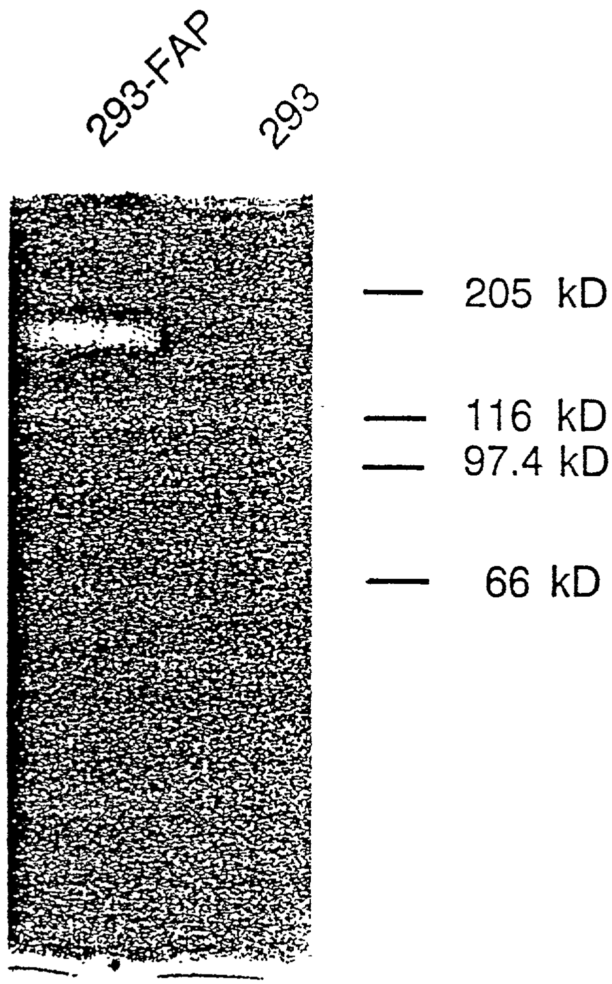
```
FAP     1 MKTWVKIVFGV*ATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILN   49
CD26    1 ---PW-VLL-LLGAA-LVTIITVPV--LNKGTDDATADSRKTY--T-Y-K   50

FAP    50 GTFSYKTFFPNWISGQEYLHQSADNNIVLYNIETGQSYTILSNRTMKSV*   98
CD26   51 N-YRL-LYSLR---DH---YKQ*E---LVF-A-Y-N-SVF-E-S-FDEFG   99

FAP    99 *NASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELP  147
CD26  100 HSIND-SI---G--IL--YN-V-Q--H----S-D----NKRQLITEERI-  149
                                                    fap-1
FAP   148 RPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIP  197
CD26  150 NNT-WVT-----H-----WN-D--V-IE-NL-SYR--WT-K-DI-Y---T  199
                                   fap-2
FAP   198 DWVYEEEMLPTKYALWWSPNGKFLAYAEFNDKDIPVIAYSYYGDE**QYP  245
CD26  200 -------VFSAYS--------T-----Q---TEV-L-E--F-S--SL---  249

FAP   246 RTINIPYPKAGAKNPVVRIFIIDT***TYPAYVGPQEVPVPAMIASSDYY  292
CD26  250 K-VRV-------V--T-KF-VVN-DSLSSVTNATSIQITA--SMLIG-H-  299

FAP   293 FSWLTWVTDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHIEES  342
CD26  300 LCDV--A-Q--IS----R-I--Y--MD---YD-SSGR-N-LVARQ---M-  349

FAP   343 RTGWAGGFFVSRPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITS  392
CD26  350 T---V-R-RP-E-H-TL-GN-F---I-NEE--R--C-FQIDKKDCTF--K  399

FAP   393 GKWEAINIFRVTQDSLFYSSNEFEEYPGRRNIYRISIGSYPPSKKCVTCH  442
CD26  400 -T--V-G-EAL-S-Y-Y-I---YKGM--G--L-K-QLSD-T*KVT-LS-E  448

FAP   443 LRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENK  492
CD26  449 -NP------SV---KE----Q-R-S---L-LY---SSVN-KGLRV--D-S  498
                                  fap-3
FAP   493 ELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKKYPLLIQVYG  542
CD26  499 A-DKM-Q-V-M-SKKLDFIILN-TKF--Q-----H--K-------LD--A  548

FAP   543 GPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKL  592
CD26  549 -----KADT--RL--AT----T-NIIV-SF----SGY----IMH-IN-R-  598

FAP   593 GVYEVEDQITAVRKFIEMGFIDEKRIAIWGWSYEIRFITGPCIWNWSFQM  642
CD26  599 -TF------E-A-Q-SK---V-N----------GGYVTSMVLGSGSVGFK  648

FAP   643 WYSSGSSLQLGILRVCLHRE*IHGSPNKDDNLEHYKNSTVMARAEYFRNV  691
CD26  649 CGIAVAPVSRWEYYDSVYT-RYM-L-TPE---D--R-----S---N-KQ-  698

FAP   692 DYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTN  741
CD26  699 E---------------Q----S----DVG--------T-ED--IASSTAH  748

FAP   742 *HLYTHMTHFLKQCFSLSD
CD26  749 Q-I----S--I------P
```

FIG. 2

	Breast Cancer	MFH	Healing Wound	Renal Cancer
FAPα	+ A	+ C	+ E	− G
CD26	− B	− D	+ F	+ H

Immunohistochemistry (See Kodachromes)

FIG. 3



DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My resident, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF, the specification of which

( ) is attached hereto.
(X) was filed on March 18, 1996 as Application Serial No. 08/619,280 and was amended on (1) ______, (2) ______ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, 1.56(a).

Foreign Priority Applications

I hereby claim foreign priority benefits under Title 35, United States Code 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	Yes ( ) No ( )
(Number)	(Country)	(Day/Month/Year Filed)	Yes ( ) No ( )

U.S. Priority Applications

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Applic. Serial No.)	(Filing Date)	(Status-patented/pending/abandoned)
08/230,491	April 20, 1994	Pending

Power of Attorney

I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: John E. Lynch, Reg. No. 20,940; Peter F. Felfe, Reg. No. 20,297; Alfred H. Hemingway, Jr., Reg. No. 26,736; Vincent M. Fazzari, Reg. No. 26,879; Norman D. Hanson, Reg. No. 30,946; Walter G. Weissenberger, Reg. No. 17,344; F. Brice Faller, Reg. No. 29,532; Andrew L. Tiajoloff, Reg. No. 31,575; John P. Luther, Reg. No. 32,261; John A. Bauer, Reg. No. 32,554 and Patricia A. Pasqualini, Reg. No. 34,894, my attorneys with full power of substitution and revocation. Address all telephone calls to Norman D. Hanson, at (212) 688-9200. Address all correspondence to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My resident, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF, the specification of which

( ) is attached hereto.
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(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
			Yes ( ) No ( )
			Yes ( ) No ( )

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(Applic. Serial No.)	(Filing Date)	(Status-patented/pending/abandoned)
08/230,491	April 20, 1994	Pending

Power of Attorney

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(ii) TITLE OF INVENTION: ISOLATED DIMERIC FIBROBLAST ACTIVATION PROTEIN ALPHA, AND USES THEREOF

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(v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 3.5 inch, 2.0 MB storage
- (B) COMPUTER: IBM PS/2
- (C) OPERATING SYSTEM: PC-DOS
- (D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/619,280
- (B) FILING DATE: 18-MARCH-1996
- (C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/230,491
- (B) FILING DATE: 20-APRIL-1994

(viii) ATTORNEY/AGENT INFORMATION:
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- (B) REGISTRATION NUMBER: 30,946
- (C) REFERENCE/DOCKET NUMBER: LUD 5330.1

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2815 Base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ. ID NO: 1:

```
AAGAACGCCC CCAAAATCTG TTTCTAATTT TACAGAAATC TTTTGAAACT TGGCACGGTA   60
TTCAAAAGTC CGTGGAAAGA AAAAAACCTT GTCCTGGCTT CAGCTTCCAA CTACAAAGAC  120
AGACTTGGTC CTTTTCAACG GTTTTCACAG ATCCAGTGAC CCACGCTCTG AAGACAGAAT  180
TAGCTAACTT TCAAAAACAT CTGGAAAAAT GAAGACTTGG GTAAAAATCG TATTTGGAGT  240
TGCCACCTCT GCTGTGCTTG CCTTATTGGT GATGTGCATT GTCTTACGCC CTTCAAGAGT  300
TCATAACTCT GAAGAAAATA CAATGAGAGC ACTCACACTG AAGGATATTT TAAATGGAAC  360
ATTTTCTTAT AAAACATTTT TTCCAAACTG GATTTCAGGA CAAGAATATC TTCATCAATC  420
TGCAGATAAC AATATAGTAC TTTATAATAT TGAAACAGGA CAATCATATA CCATTTTGAG  480
TAATAGAACC ATGAAAAGTG TGAATGCTTC AAATTACGGC TTATCACCTG ATCGGCAATT  540
TGTATATCTA GAAAGTGATT ATTCAAAGCT TTGGAGATAC TCTTACACAG CAACATATTA  600
CATCTATGAC CTTAGCAATG GAGAATTTGT AAGAGGAAAT GAGCTTCCTC GTCCAATTCA  660
GTATTTATGC TGGTCGCCTG TTGGGAGTAA ATTAGCATAT GTCTATCAAA ACAATATCTA  720
TTTGAAACAA AGACCAGGAG ATCCACCTTT TCAAATAACA TTTAATGGAA GAGAAAATAA  780
AATATTTAAT GGAATCCCAG ACTGGGTTTA TGAAGAGGAA ATGCTTCCTA CAAAATATGC  840
TCTCTGGTGG TCTCCTAATG GAAAATTTTT GGCATATGCG GAATTTAATG ATAAGGATAT  900
ACCAGTTATT GCCTATTCCT ATTATGGCGA TGAACAATAT CCTAGAACAA TAAATATTCC  960
ATACCCAAAG GCTGGAGCTA AGAATCCCGT TGTTCGGATA TTTATTATCG ATACCACTTA 1020
CCCTGCGTAT GTAGGTCCCC AGGAAGTGCC TGTTCCAGCA ATGATAGCCT CAAGTGATTA 1080
TTATTTCAGT TGGCTCACGT GGGTTACTGA TGAACGAGTA TGTTTGCAGT GGCTAAAAAG 1140
AGTCCAGAAT GTTTCGGTCC TGTCTATATG TGACTTCAGG GAAGACTGGC AGACATGGGA 1200
TTGTCCAAAG ACCCAGGAGC ATATAGAAGA AAGCAGAACT GGATGGGCTG GTGGATTCTT 1260
TGTTTCAAGA CCAGTTTTCA GCTATGATGC CATTTCGTAC TACAAAATAT TTAGTGACAA 1320
GGATGGCTAC AAACATATTC ACTATATCAA AGACACTGTG GAAAATGCTA TTCAAATTAC 1380
AAGTGGCAAG TGGGAGGCCA TAAATATATT CAGAGTAACA CAGGATTCAC TGTTTTATTC 1440
TAGCAATGAA TTTGAAGAAT ACCCTGGAAG AAGAAACATC TACAGAATTA GCATTGGAAG 1500
CTATCCTCCA AGCAAGAAGT GTGTTACTTG CCATCTAAGG AAAGAAAGGT GCCAATATTA 1560
CACAGCAAGT TTCAGCGACT ACGCCAAGTA CTATGCACTT GTCTGCTACG GCCCAGGCAT 1620
CCCCATTTCC ACCCTTCATG ATGGACGCAC TGATCAAGAA ATTAAAATCC TGGAAGAAAA 1680
CAAGGAATTG GAAAATGCTT TGAAAAATAT CCAGCTGCCT AAAGAGGAAA TTAAGAAACT 1740
TGAAGTAGAT GAAATTACTT TATGGTACAA GATGATTCTT CCTCCTCAAT TTGACAGATC 1800
AAAGAAGTAT CCCTTGCTAA TTCAAGTGTA TGGTGGTCCC TGCAGTCAGA GTGTAAGGTC 1860
TGTATTTGCT GTTAATTGGA TATCTTATCT TGCAAGTAAG GAAGGGATGG TCATTGCCTT 1920
GGTGGATGGT CGAGGAACAG CTTTCCAAGG TGACAAACTC CTCTATGCAG TGTATCGAAA 1980
GCTGGGTGTT TATGAAGTTG AAGACCAGAT TACAGCTGTC AGAAAATTCA TAGAAATGGG 2040
TTTCATTGAT GAAAAAAGAA TAGCCATATG GGGCTGGTCC TATGGAGGAT ACGTTTCATC 2100
ACTGGCCCTT GCATCTGGAA CTGGTCTTTT CAAATGTGGT ATAGCAGTGG CTCCAGTCTC 2160
CAGCTGGGAA TATTACGCGT CTGTCTACAC AGAGAGATTC ATGGGTCTCC CAACAAAGGA 2220
TGATAATCTT GAGCACTATA AGAATTCAAC TGTGATGGCA AGAGCAGAAT ATTTCAGAAA 2280
TGTAGACTAT CTTCTCATCC ACGGAACAGC AGATGATAAT GTGCACTTTC AAAACTCAGC 2340
ACAGATTGCT AAAGCTCTGG TTAATGCACA AGTGGATTTC CAGGCAATGT GGTACTCTGA 2400
CCAGAACCAC GGCTTATCCG GCCTGTCCAC GAACCACTTA TACACCCACA TGACCCACTT 2460
CCTAAAGCAG TGTTTCTCTT TGTCAGACTA AAAACGATGC AGATGCAAGC CTGTATCAGA 2520
ATCTGAAAAC CTTATATAAA CCCCTCAGAC AGTTTGCTTA TTTTATTTTT TATGTTGTAA 2580
AATGCTAGTA TAAACAAACA AATTAATGTT GTTCTAAAGG CTGTTAAAAA AAAGATGAGG 2640
ACTCAGAAGT TCAAGCTAAA TATTGTTTAC ATTTTCTGGT ACTCTGTGAA AGAAGAGAAA 2700
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AGGGAGTCAT GCATTTTGCT TTGGACACAG TGTTTTATCA CCTGTTCATT TGAAGAAAAA 2760
TAATAAAGTC AGAAGTTCAA AAAAAAAAAA AAAAAAAAAA AAAGCGGCCG CTCGA      2815
```

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 760 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Lys Thr Trp Val Lys Ile Val Phe Gly Val Ala Thr Ser Ala Val
                 5                  10                  15

Leu Ala Leu Leu Val Met Cys Ile Val Leu Arg Pro Ser Arg Val His
            20                  25                  30

Asn Ser Glu Glu Asn Thr Met Arg Ala Leu Thr Leu Lys Asp Ile Leu
        35                  40                  45

Asn Gly Thr Phe Ser Tyr Lys Thr Phe Phe Pro Asn Trp Ile Ser Gly
    50                  55                  60

Gln Glu Tyr Leu His Gln Ser Ala Asp Asn Asn Ile Val Leu Tyr Asn
65                  70                  75                  80

Ile Glu Thr Gly Gln Ser Tyr Thr Ile Leu Ser Asn Arg Thr Met Lys
                85                  90                  95

Ser Val Asn Ala Ser Asn Tyr Gly Leu Ser Pro Asp Arg Gln Phe Val
            100                 105                 110

Tyr Leu Glu Ser Asp Tyr Ser Lys Leu Trp Arg Tyr Ser Tyr Thr Ala
        115                 120                 125

Thr Tyr Tyr Ile Tyr Asp Leu Ser Asn Gly Glu Phe Val Arg Gly Asn
    130                 135                 140

Glu Leu Pro Arg Pro Ile Gln Tyr Leu Cys Trp Ser Pro Val Gly Ser
145                 150                 155                 160

Lys Leu Ala Tyr Val Tyr Gln Asn Asn Ile Tyr Leu Lys Gln Arg Pro
                165                 170                 175

Gly Asp Pro Pro Phe Gln Ile Thr Phe Asn Gly Arg Glu Asn Lys Ile
            180                 185                 190

Phe Asn Gly Ile Pro Asp Trp Val Tyr Glu Glu Glu Met Leu Pro Thr
        195                 200                 205

Lys Tyr Ala Leu Trp Trp Ser Pro Asn Gly Lys Phe Leu Ala Tyr Ala
    210                 215                 220
```

Glu Phe Asn Asp Lys Asp Ile Pro Val Ile Ala Tyr Ser Tyr Tyr Gly
225 230 235 240

Asp Glu Gln Tyr Pro Arg Thr Ile Asn Ile Pro Tyr Pro Lys Ala Gly
245 250 255

Ala Lys Asn Pro Val Val Arg Ile Phe Ile Ile Asp Thr Thr Tyr Pro
260 265 270

Ala Tyr Val Gly Pro Gln Glu Val Pro Val Pro Ala Met Ile Ala Ser
275 280 285

Ser Asp Tyr Tyr Phe Ser Trp Leu Thr Trp Val Thr Asp Glu Arg Val
290 295 300

Cys Leu Gln Trp Leu Lys Arg Val Gln Asn Val Ser Val Leu Ser Ile
305 310 315 320

Cys Asp Phe Arg Glu Asp Trp Gln Thr Trp Asp Cys Pro Lys Thr Gln
325 330 335

Glu His Ile Glu Glu Ser Arg Thr Gly Trp Ala Gly Gly Phe Phe Val
340 345 350

Ser Arg Pro Val Phe Ser Tyr Asp Ala Ile Ser Tyr Tyr Lys Ile Phe
355 360 365

Ser Asp Lys Asp Gly Tyr Lys His Ile His Tyr Ile Lys Asp Thr Val
370 375 380

Glu Asn Ala Ile Gln Ile Thr Ser Gly Lys Trp Glu Ala Ile Asn Ile
385 390 395 400

Phe Arg Val Thr Gln Asp Ser Leu Phe Tyr Ser Ser Asn Glu Phe Glu
405 410 415

Glu Tyr Pro Gly Arg Arg Asn Ile Tyr Arg Ile Ser Ile Gly Ser Tyr
420 425 430

Pro Pro Ser Lys Lys Cys Val Thr Cys His Leu Arg Lys Glu Arg Cys
435 440 445

Gln Tyr Tyr Thr Ala Ser Phe Ser Asp Tyr Ala Lys Tyr Tyr Ala Leu
450 455 460

Val Cys Tyr Gly Pro Gly Ile Pro Ile Ser Thr Leu His Asp Gly Arg
465 470 475 480

Thr Asp Gln Glu Ile Lys Ile Leu Glu Glu Asn Lys Glu Leu Glu Asn
485 490 495

Ala Leu Lys Asn Ile Gln Leu Pro Lys Glu Glu Ile Lys Lys Leu Glu
500 505 510

Val Asp Glu Ile Thr Leu Trp Tyr Lys Met Ile Leu Pro Pro Gln Phe
515 520 525

Asp Arg Ser Lys Lys Tyr Pro Leu Leu Ile Gln Val Tyr Gly Gly Pro
530 535 540

Cys Ser Gln Ser Val Arg Ser Val Phe Ala Val Asn Trp Ile Ser Tyr
545 550 555 560

Leu Ala Ser Lys Glu Gly Met Val Ile Ala Leu Val Asp Gly Arg Gly
565 570 575

Thr Ala Phe Gln Gly Asp Lys Leu Leu Tyr Ala Val Tyr Arg Lys Leu
580 585 590

Gly Val Tyr Glu Val Glu Asp Gln Ile Thr Ala Val Arg Lys Phe Ile
595 600 605

Glu Met Gly Phe Ile Asp Glu Lys Arg Ile Ala Ile Trp Gly Trp Ser
610 615 620

Tyr Gly Gly Tyr Val Ser Ser Leu Ala Leu Ala Ser Gly Thr Gly Leu
625 630 635 640

Phe Lys Cys Gly Ile Ala Val Ala Pro Val Ser Ser Trp Glu Tyr Tyr
645 650 655

Ala Ser Val Tyr Thr Glu Arg Phe Met Gly Leu Pro Thr Lys Asp Asp
660 665 670

Asn Leu Glu His Tyr Lys Asn Ser Thr Val Met Ala Arg Ala Glu Tyr
675 680 685

Phe Arg Asn Val Asp Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn
690 695 700

Val His Phe Gln Asn Ser Ala Gln Ile Ala Lys Ala Leu Val Asn Ala
705 710 715 720

Gln Val Asp Phe Gln Ala Met Trp Tyr Ser Asp Gln Asn His Gly Leu
725 730 735

Ser Gly Leu Ser Thr Asn His Leu Tyr Thr His Met Thr His Phe Leu
740 745 750

Lys Gln Cys Phe Ser Leu Ser Asp
755 760

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 766 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
5 10 15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr
20 25 30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
35 40 45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
50 55 60

Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn
65 70 75 80

Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp
85 90 95

Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln
100 105 110

Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr
115 120 125

Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr
130 135 140

Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val
145 150 155 165

Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile
170 175 180

Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp
185 190 195

Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe
200 205 210

Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala
215 220 225

Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe
230 235 240 245

```
Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr
                250                 255                 260

Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn
            265                 270                 275

Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr
        280                 285                 290

Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr
    295                 300                 305

Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln
310                 315                 320                 325

Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg
                330                 335                 340

Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly
            345                 350                 355

Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly
        360                 365                 370

Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile
    375                 380                 385

Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly
390                 395                 400                 405

Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr
                410                 415                 420

Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr
            425                 430                 435

Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu
        440                 445                 450

Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu
    455                 460                 460

Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr
465                 470                 475                 480

Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp
                485                 490                 495

Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys
            500                 505                 510

Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met
        515                 520                 525
```

```
Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu
    530                 535                 540

Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg
545                 550                 555                 560

Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala
                565                 570                 575

Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His
            580                 585                 590

Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp Gln Ile Glu
        595                 600                 605

Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn Lys Arg Ile
    610                 615                 620

Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser Met Val Leu
625                 630                 635                 640

Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val Ala Pro Val
                645                 650                 655

Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg Tyr Met Gly
            660                 665                 670

Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn Ser Thr Val
        675                 680                 685

Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu Leu Ile His
    690                 695                 700

Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala Gln Ile Ser
705                 710                 715                 720

Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met Trp Tyr Thr
                725                 730                 735

Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln His Ile Tyr
            740                 745                 750

Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu Pro
        755                 760                 765
```

(2) INFORMATION FOR SEQ ID NO: 4:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ix) FEATURE:
        (D) OTHER INFORMATION: The first Xaa is either Trp or Phe.
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Xaa Gly Trp Ser Tyr Gly Gly
                5

(2) INFORMATION FOR SEQ ID NO: 5:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Phe Gly Lys Asp Tyr Gly Gly
                5

(2) INFORMATION FOR SEQ ID NO: 6:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (ix) FEATURE:
        (D) OTHER INFORMATION: Xaa is either Ala or Gly
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly Thr Xaa Asp Asp Asn Val
                5

(2) INFORMATION FOR SEQ ID NO: 7:
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ala Gln Asn His Gly Leu Ser
                5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION:
The first Xaa is Glu or Ser. When the first Xaa is Glu, the second Xaa is Gly and the third is Ala. When the first Xaa is Ser, the second Xaa is Ser, and the third Xaa is Arg.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Asp Xaa Asp His Xaa Ile Xaa
                5
```

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION:
Xaa is Pro or Ala.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Xaa Thr Ala Asp Glu Lys Ile
                5
```

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION:
Xaa is Thr, His or Ser.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Asp Glu Ser His Tyr Phe Xaa
                5
```